by others. In my opinion, the central conclusion from this study is the positive feedback loop between α-synuclein accumulation and GCase dysfunction; obtaining data to support or refute this idea will be key to the progress of understanding why *GBA* mutations influence the risk of Parkinson's disease in the next few years.

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A YY1 Bridge for X Inactivation

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Xist RNA inactivates one mammalian X chromosome (the Xi) by associating with it in *cis*. The mechanism of this interaction is unresolved. Jeon and Lee (2011) now show that YY1 binds both Xist RNA and DNA, thereby providing a mechanism to anchor Xist to the Xi and facilitate X chromosome inactivation.

Inactivation of one X chromosome in female mammals is required to equalize gene dosage between males and females. This process is regulated by numerous long noncoding RNAs (lncRNAs) with Xist paramount among them (Lee, 2009). Initiation of X chromosome inactivation (XCI) requires the expression of *Xist* exclusively from the future inactive X (Xi), where it will ultimately coat the entire X chromosome and induce chromatin modifications to silence genes encoded on the Xi. How the Xist RNA becomes associated with and coats the future Xi has remained an enigma. In this issue of *Cell*, Jeon and Lee report a clever and somewhat surprising set of studies indicating that a protein, YY1, associates with the *Xist* gene locus and the Xist RNA; this raises the possibility that YY1 has a direct role in bridging the Xist RNA and the Xi (Jeon and Lee, 2011).

Using differentiated, post-XCI cell lines (immortalized mouse embryonic fibroblasts [MEFs]), the authors set out to study *Xist* expression from an inducible transgene with the goal of elucidating how Xist RNA becomes tethered to its chromosome of origin. They tested a model in which a specific *Xist* DNA sequence is required to tether Xist RNA in *cis*, possibly serving as the nucleation site for the spread of Xist RNA along the future Xi. By introducing an *Xist* transgene into the genome of differentiated cells, the *Xist* DNA was not exposed to chromatin modifications arising during developmental programming. RNA FISH and RT-PCR assays were used to discriminate between endogenous and transgenic Xist RNA and measure their relative expression in female cells. As expected, upon induction, transgenic Xist RNA was highly expressed and localized in *cis* to the transgene. Surprisingly, in female transgenic lines, RNA FISH revealed that the normally abundant Xist RNA associated with the endogenous Xi was diminished. Neither Xist instability nor reduced transcription of endogenous Xist explained these results. Rather, endogenous Xist RNA was migrating (in *trans*) to the transgenic *Xist* DNA locus. This was a surprising finding as Xist had never before been seen to migrate between chromosomes and so was thought to act only in *cis*.

The observation of diffusible Xist provided a means to dissect further the *Xist* DNA and RNA sequences responsible

for tethering Xist RNA to the *Xist* locus using mutant *Xist* transgenic female MEFs. Analysis of these mutant lines suggested that the three Yin Yang 1 (YY1) binding sites within the exon 1 Repeat F region of the *Xist* gene are critical to tether Xist RNA. ChIP analysis showed the association of YY1 with these sites in vivo. Consistently, YY1 knockdown in the female MEFs and also in differentiating female embryonic stem cell lines abrogated Xist RNA association with the Xi.

YY1 is well-appreciated as a DNA-binding transcription factor and as a player in controlling XCI (Donohoe et al., 2007), and it has also been reported to bind structurally divergent RNA species, including maternal RNAs in *Xenopus* oocytes (Belak et al., 2008). Looking more closely at its association with Xist, the authors established that YY1 binds to Xist RNA directly through the RepC RNA sequence (see Figure 1) in vivo (RNA immunoprecipitation) and in vitro (RNA pulldown). These results nicely support an earlier study implicating Xist RepC RNA sequence in RNA coating of the Xi (Beletskii et al., 2001). Thus, the authors describe dual binding properties for YY1 that support a model in which YY1 localizes Xist RNA to the *Xist* gene locus. Having demonstrated that Xist RNA associates with YY1, Jeon and Lee provide further incentive to define the specific RNA sequence and YY1 domain required for this interaction. The authors propose that the zinc finger domain of YY1 may simultaneously bind both Xist RNA and DNA, although the protein appears to bind to different sequence motifs in the two polymers.

YY1's role in X inactivation is likely to be multifaceted. It can recruit Polycomb Group-repressive complexes to chromatin (validated in *Drosophila* but questioned in mammals), which may also contribute to PRC2 recruitment to the Xi (Jeon and Lee, 2011; Wilkinson et al., 2006). Additionally, other YY1-binding sites on the X chromosome may participate in XCI. For example, there are clustered YY1 sites downstream of *Xist* that are proposed to be required for activation of the *Tsix* gene (Donohoe et al., 2007). This lncRNA is important on the future active X chromosome (Xa) to repress *Xist* expression. These clusters of binding sites are reminiscent of YY1 association across the immunoglobulin heavy-chain locus that facilitates $V_HD_HJ_H$ recombination. Thus, YY1-binding sites across the *Xist*/*Tsix* locus may work together to facilitate X inactivation (Liu et al., 2007). In summary, YY1 may function in XCI in multiple capacities, and careful follow-up will be required to tease apart these roles.

In addition to YY1, the authors considered the possibility that CTCF was involved in Xist localization. This ubiquitous and multifunctional protein was previously shown by the authors to cooperate with YY1 in activation of *Tsix* on the Xa (Donohoe et al., 2007). In the present study, however, the results of *Ctcf* knockdown experiments were not consistent with CTCF contributing to Xist association with the Xi (Jeon and Lee, 2011).

Xist may offer a model system for defining the function of other lncRNAs (Lee, 2009), and the current study demonstrates the importance of defining RNA-protein interactions to understanding lncRNA function. An algorithm (catRAPID) that predicts protein-RNA association (Bellucci et al., 2011) successfully predicted the previously reported Xist RepA RNA-PRC2 association (Zhao et al., 2008), and it will be of interest to see whether catRAPID also identifies the association between Xist RepC RNA and YY1 (Jeon and Lee, 2011), as well as other proposed Xist RNA-protein interactions (Arthold et al., 2011). If so, catRAPID could gain traction as a useful tool in identifying proteins required for the function of other lncRNAs. The question also arises as to whether lessons learned from the study of Xist-protein interactions in vivo and in silico can be extrapolated to other lncRNAs, particularly those involved in silencing at imprinted loci.

In summary, Jeon and Lee have shown that YY1 associates with both *Xist* DNA and RNA. They propose that these associations are critical to tether Xist RNA to the future Xi and to nucleate the coating of Xist RNA along the Xi. They also show that YY1 binds a specific *Xist* DNA sequence and that YY1 associates with a specific Xist RNA sequence (RepC) (see Figure 1). YY1, however, is a ubiquitous protein that is critical for development and likely regulates many processes. The generation of endogenous *Xist* mutations in ES cells (and preferably in mice) will ultimately define YY1's role in nucleating Xist RNA association with the future Xi.

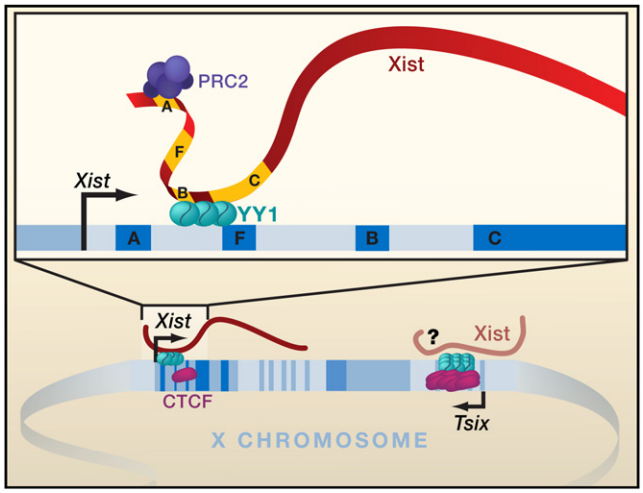

Figure 1. YY1 Associates with Both Xist RNA and DNA, Facilitating X Inactivation

Xist RNA plays a critical role in X chromosome inactivation in females, coating the Xi and directly recruiting silencing factors such as PRC2. Jeon and Lee now demonstrate that Xist RNA is tethered to the Xi by Yin Yang 1 (YY1), which binds to distinct sites at the *Xist* gene locus and within the RNA. In contrast, CTCF sites do not appear to be involved in tethering despite close proximity to the critical YY1 sites. A large cluster of YY1 and CTCF sites within *Tsix* was previously shown to be important on the Xa (Donohoe et al., 2007); however, an additional role on the Xi has not been ruled out. (Figure not to scale.)

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A Flip Turn for Membrane Protein Insertion

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The transmembrane domains in a membrane protein must be recognized and correctly oriented before their insertion into the lipid bilayer. Devaraneni et al. (2011) generate snapshots at different stages of membrane protein biogenesis, revealing a dynamic set of steps that imply an unexpectedly flexible membrane insertion machinery.

Integral membrane proteins typically acquire their topology and insert into the membrane cotranslationally as the polypeptide is emerging from a ribosome. These critical biosynthetic events occur at a membrane-embedded translocon through which the nascent membrane protein is translocated (Osborne et al., 2005). The ribosome translocon complex (RTC) is responsible for recognizing the transmembrane domains (TMDs) of membrane proteins, orienting them in the correct topology and inserting them into the lipid bilayer. Whereas the sequence features of a TMD and its flanking domains that influence topology have been extensively characterized (von Heijne, 2006), far less is known about how this information is decoded by the RTC to ensure accurate membrane protein topogenesis. In this issue of *Cell*, Devaraneni et al. (2011) demonstrate that both a TMD and the RTC can experience dynamic conformational changes during topogenesis, providing new insights into the complex process of membrane protein biogenesis.

A mechanistic understanding of TMD insertion requires high-resolution information about RTC structure, the nascent polypeptide within the RTC, and how their relative configurations change over time. Because these events occur cotranslationally, the process is necessarily rapid and dynamic, severely complicating most methods of analysis. The traditional way to circumvent this temporal problem is by assembling ''stalled'' translocation intermediates of defined polypeptide lengths in vitro. Examination of these presumptive intermediates by structural, biochemical, and biophysical methods has provided much of our current insights into protein translocation and membrane insertion (Osborne et al., 2005).

However, cohesive models for membrane protein insertion have been challenging to derive because different studies have employed different assays, probes, and substrates, yielding sometimes contradictory findings. To tackle this problem, Devaraneni et al. (2011) simultaneously employ multiple types of probes (Figure 1A) on successively longer RTCs of a model protein with a TMD that achieves the type II orientation (with the N terminus facing the cytosol and C terminus translocated across the membrane). Each probe is designed to assess the location of specific residues in the nascent chain relative to the RTC, the membrane, or both. Though not all assays are informative at every length, this extensive and systematic strategy nonetheless provides ''snapshots'' of each nascent chain-RTC intermediate. When these snapshots are stitched together, the resulting ''stop motion animation'' allows the authors to propose a model for how a single-spanning membrane protein inserts into the lipid bilayer (Figure 1B).

The model for insertion of a type II TMD consists of four coordinated and dynamic steps. First, at short nascent chain lengths, the TMD initially inserts in a